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Version 2

Immunostaining on Paraffin Sections of Fly Heads V.2



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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes how to perform immunostaining on paraffin sections of fly heads.

Troubleshooting



- 1 Fix fly heads overnight in 4% formalin.
- 2 Embed in paraffin.
- 3 Cut 4 micron sections. Dry at 42 °C Overnight .
- 4 Deparaffinize and bring through ethanols to water (xylene x 2, 100% ethanol x 4, tap H₂O x 2).
- 5 Microwave slides in 10 millimolar (mM) sodium citrate for 00:15:00 . 35m
Cool 00:20:00 .

Stock: 100 millimolar (mM) sodium citrate, pH 6.0
Use at least 1 L of citrate solution in large glass box to avoid drying.
- 6 Block in PBST (PBS with 0.3% Triton) with 2% dry milk for 00:30:00 to 1h 30m
Stock: 10X PBS
- 7 Incubate with primary antibody Overnight at Room temperature .
- 8 Wash 3 x in PBST.
- 9 Incubate with appropriate biotinylated secondary (for immunohistochemistry) or fluorescent secondary (for immunofluorescence) antibody at 1:200 in PBST + milk for 01:00:00 at Room temperature . 2h
For immunohistochemistry incubate in ABC reagent (Vector) for 01:00:00 at Room temperature .



- 10 Rinse 3 x PBST.
- 11 Mount slides with antifading medium for immunofluorescence or dehydrate through ethanol series and xylenes and mount in Permount for immunohistochemistry.